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# Pathological anatomy of experimental leukemia contracted through introduction of benzole extracts from organs of patients who died of leukemia

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In the course of the present work, we took on the task of studying the pathological anatomy of experimental leukemia contracted through the introduction into animals of benzol extracts from various organs of patients who died of leukemia, to ascertain its morphological criteria, and also to investigate the dynamics of initial transformations.

Experimental leukemia which we studied was obtained from the pathophysiological laboratory by M O Rauschenbach. In his study[1] is set forth in detail the methodology of receiving leukemia, the results of examining the peripheral blood, and a characterization of the breed of experimental animal which we studied is also given.

The mice were divided into six groups according to the introduction of benzol extracts from the organs of patients: (1) chronic myelosis (2) chronic lymphadenosis (3) acute leukemia (4) myeloma (5) cancer of the stomach. In the sixth group were put mice into whom was introduced an extract from organs of healthy persons who died of street injuries. The last two groups were control groups.

In all groups some of the animals died in the course of the first two or three months of the experiment. We noticed in them numerous focal necroses in inner organs, thromboses of blood vessels, focal hyperplasia of the cells of the reticuloendothelial system, at times amiloidosis of spleen and liver. The changes enumerated are connected with toxic effects of benzol extract.

In the first group were mice into whom was introduced benzol extract from the organs of four patients who died of chronic myelosis. Among 75 animals studied, which lived four months or longer from the beginning of the study, in the inner organs and marrow of four we found changes which permit us to speak of the development of leukemia.

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(1) See the Article of M O Rauschenbach "About the blastomatogenous substrate in the tissues of patients with leukemia" published in the same issue of the journal.

Mouse No. 1: Benzol extract from the liver of a patient who died of chronic myelosis was introduced. Killed four months and 10 days after the beginning of the experiment. The blood composition without changes.

In autopsy it was ascertained that inner organs were not enlarged but have a normal appearance. In the region of the loins in the place where the extract was introduced, there was a tumorous swelling the size of a pigeon's ear, which in cross-section was grey with large nidi of necrosis.

Microscopic examination. Liver. Around the central veins and nidi in the lumens of the capillaries there are affluxes of large round and oval cells with weakly basofile protoplasm and a strong light kernel (nucleus ?) (cells of the type of myelitis); in some of the cells the kernel has the form of a ring (myelocytes). There can be noticed nidi of destruction of liver cells.

Spleen. Strong follicles, in the pulp hyperplasis and focal myelosis. Eridothelium of sinuses swollen. A small number of megacariocytes.

Lymphatic know under the armpit. Diffuse hyperplasis with full obliteration of the pattern. Under the capsule, foci from the large myeloid cell described above. A small focal accumulation of the same cells around the blood vessels in lungs and kidneys.

Marrow of the hip. Polymorphous state. Many lymphoid elements in the midst of which are found groups of myeloblasts, myelocytes and undifferentiated cells of the type of hemocytoblasts.

Swelling In parts not subject to necrosis, composed of atypical long cells of irregular size with prolonged centers. Gigantic cells are found. The centers are rich in chromatine, incorrect deformed form, many figures of difision. The cells described form clusters woven together in various directions which corresponds with the picture of spindlecellular sarcoma. The presence of nidi of myeloid metaplasis in the marrow and in the inner organs testifies to a developing leukemic process.

Mouse No. 2: Benzol extracts introduced from liver of patient who died of chronic myelosis. Killed after four months and 23 days after the beginning of the experiment.

Blood Analysis. Leukocytes 57,800, myeloblasts 2 per cent

Microscopically changes in the inner organs could not be noticed. In the region of the loins a tumor the size of a pigeon's ear.

Microscopic examination. Liver. Lumina of the capillaries widened, filled with myeloid elements (ill. 1), which in places form large groups with an obliteration of the pattern of lobules. Kupfer cells swollen. Here and there in the capillaries megacariocytes are found. Small infiltrates from myeloid cells are also present along the course of glisson capsule.

Spleen. Follicles well developed, in the center of cells of pulp myeloid elements predominate.

Lymphatic Knot. Focal myelosis.

Marrow. Consists for the main part of cells with myeloid order, starting with myeloblasts and ending with segmented leukocytes. Myelocytes predominate. Great quantity of megacariocytes; many of them have a picnotic center.

Tumor. Has the structure of spindle-cellular sarcoma.

Thus, Mouse No 2 developed leukemia, with a predominance of myelocytes in the midst of myeloid elements.

Mouse No 3. Benzol extract from the liver of a patient who died of chronic myelosis was introduced. Killed after 10 months 18 days after the beginning of the experiment.

In opening the abdominal cavity, a great quantity of blood fluid was found.

Microscopic study. The liver enlarged. In the place where the extract was introduced, a tumorous swelling. In microscopic examination there was found in the liver a sharply defined leukemic infiltration, myelosis of the spleen and lymphatic knots, myeloid metaplasis of the marrow of the hip. The swelling had the structure of a spindle-cellular sarcoma.

Analogous changes developed in Mouse No 2, into whom was introduced benzol extract from the liver of a patient who died of chronic myelosis. Killed after 10 months and 20 days after the beginning of the experiments. In peripheral blood, no changes noticed.

In subsequent transfers both of emulsion of the swelling, as well as the emulsion of inner organs and blood of leukemic animals, in a number of cases there was the development of myelosis with pathoanatomical changes analogous to the changes described above.

With the introduction of benzol extract into mice from the organs of patients who died of chronic myelosis, we observed the development of myelosis, with the predominance, amidst the bloodformative cells, of myelocytes.

In the second group belonged the mice into whom was introduced benzol extract from organs of three patients who died of chronic lymphadenosis.

In a few of the 36 mice studied in the group, there were noticed accumulations in the lumina of the capillaries of the liver of an increased number of cells of the lymphocyte type and moderate hyperplasis of lymphatic tissue in the spleen and lymphatic knots, otherwise leukemic changes were absent. In a number of mice there was noticed a development of tumor both in the place of introduction of the preparation and also at a distance from it.

After futther transfers, the development of leukemia was noticed. We shall give two protocols by way of example.

Mouse No 2. First Transfer. Killed after one month and 20 days after the introduction of the emulsion from the swelling (microscopic swelling had the structure of polymorphous sarcoma).

Blood Analysis. Leukocytes 77.000; myeloblasts, 3 per cent; myelocytes, 11 per cent.

During dissection of the back in the area of introduction of the emulsion a thickening the size of a pea was found. The spleen was a little enlarged.

Microscopic examination. Liver. Large nidi of neucrosis. In the parts of the preserved tissue, the pattern of the lobules erased as a result of the presence of large infiltrates from myelode cells alongthe course of glisson capsule and their accumulation in the lumina of the capillaries.

Spleen. Diffuse myelosis of pulp. Follicles preserved, shallow (ill. 2).

Lymphatic Knots. Focal myelosis.

Lungs. Capillaries of inter-veolary partitions widened, filled with myeloid cells. In remaining inner organs, changes not present.

Marrow. A definite predominance of myeloid cells, on the background of which we find a small quantity of cells of red blood. It is necessary to note that in the midst of the myeloid cells in all organs there predominate large and small myelocytes, together with which there are many segmented and hypersegmented leukocytes (ill. 3).

The thickening on the backhas the structure of spindle-cellular sarcoma.

Mouse No 5 killed after one month and two days after introduction of emulsion.

Analysis of Blood. Leukocytes 45 thousand, myeloblasts 5 per cent, myelocytes 9 per cent. Microscopically noticeable a small enlargement of the spleen. Tumor absent.

Microscopic examination. Found a pattern of myelosis of the liver, spleen, marrow and focal myelosis in the lymphatic glands. At the same time, there was amiloidosis of inner organs.

Thus, with the introduction of benzol extracts from the organs of patients who died of chronic lymphadenosis, we noticed only the development of tumors.

After subsequent transfers of emulsions of tumors, there was noticed in some of the mice the development of myelosis. Also, as with mice of the first group, with these there predominated, myelocytal type of leukemia.

In the third group we put mice into whom was introduced benzol extract from the organs of three patients who died of acute leukemia. The greatest interest in this group is in the mice into whom was introduced extract from the brain. Among 32 mice studied, three developed leukemia, two of them in conjunction with tumors; in one was noticed the simultaneous development of tumors in the place of the introduction of the extract and at a distance, and in the second there developed only the so-called distant tumor.

We notice that in all three cases in the midst of blood-creating cells there predominated the most unripe elements of myeloid kind--hemocytoblasts and myeloblasts.

Mouse No 5. Killed after five months after the beginning of the experiment.

Blood Count. Leukocytes 44 thousand; hemocytoblasts, 8 per cent; myeloblasts 6 per cent.

Microscopically, on the back there was noticed a tumorous swelling. In the area of the left kidney there is a swelling the size of a bean, partially growing through the tissue of the kidney. In cross-section the swelling is white and dry.

The spleen, the liver and the right kidney enlarged.

Microscopic examination. Liver. The pattern fully erased. On the background of large areas of amyloid there are spread groups of liver cells. Capillaries widened, filled with large, oval cells with large nuclei of the type of hemocytoblasts. In some places these cells make up large accumulations. Together with them in smaller quantity are found myeloblasts and single large myelocytes.

Spleen. Follicles preserved, shallow. In the pulp a large quantity of the same cells.

Right kidney. In the crust substance, between the canals, focal accumulations of the same undifferentiated cells of the type of hemocytoblasts.

Tumor in the region of the left kidney has the structure of spindle-cellular sarcoma, contains extensive nidi of necrosis.

Tumor on the back. The structure impossible to define as the tissue is fully necroticized.

Marrow of the hip consists of the above-described cells of hemocytoblast type.

In transfer, also, the hemocytoblast-like type of leukemia was obtained.

Thus, when benzol extracts from the organs of patients who died of acute leukemia are introduced, both in the original group and in the transfers, developments of hemocytoblasts are noticed.

In the fourth group were the mice into whom was introduced benzol extract from the liver and marrow of a patient who died of myelosis.

One of the 19 mice examined (Mouse No 7), killed after eight months 23 days after the introduction of the extract, microscopically there was found a pattern of myelosis with a predominance of myelocytes with acute myloidosis of the organs. In the thyroid gland there was hyperplasis of lymphoid tissue. In peripheral blood the quantity of leukocytes was increased to 24 thousand. Myelocytes 4 per cent. In Mouse No 1 killed after five months after the beginning of the experiment, in the abdominal cavity there were many knots of tumors, composed of cells of myelomnic type. The same cells composed the tissue of the marrow. Thus in this mouse changes of the type of diffuse myelosis took place. From Mouse No 7, through transfer, myelosis was obtained.

In the fifth group were animals into whom was introduced extract from the liver of two patients who died of cancer.

In two of the 18 mice studied microscopically, there was found myelosis with a predominance of myeloblasts, with enlargement of leukocytes in peripheral blood (to 46,200) and the appearance of immature (unripe) cells of myeloid kind (16-22 per cent).

In microscopic examination of mice into whom benzol extract was introduced from livers of persons who died of street injuries, in some we found amiloidosis and focal necrosis, connected with toxic effects of the extracts. Leukemia did not develop. Thus, of 162 mice aged over six months, which lived not less than 4-4½ months after the introduction of benzol extracts from organs of patients who died of leukemia, eight (4.9 per cent) developed leukemia.

Besides the original mice, we studied 250 mice into whom was transferred both emulsion of swelling and emulsion of inner organs and transfusion of blood of leukemic animals. Among the cases there was noticed the development of leukemia of the myelose type and hemocytosis with pathologoanatomical changes analogous to those described above. Leukemia reached the greatest intensity in three or four transfers in which myeloid metaplasis developed in a greater number of animals (approx. 80 per cent) than in the original group, and noticeably more quickly. If in the original mice the development of leukemia was observed four months or more after the beginning of the experiment, with the transfers this delay decreased to 20-30 and in single cases even to 15 days.

Thus, in the development of leukemia in mice there was observed diffuse myeloid hyperplasis of marrow, myelosis of the spleen and lymphatic glands, infiltration of myeloid elements of the liver tissue.

Comparing the changes which take place in experimental leukemia achieved through the method of introducing known synthetic cancerogenous materials (Yudins, Engelbret-Holm) with the changes developed through the introduction of benzol extracts from orgars of patients who died of leukemia, it is possible to state that in their morphology they are identical. Leukemia started in mice through the introduction of benzol extracts from the organs of patients who died of leukemia has a series of pathologoanatomical characteristics to which we have partly already referred during the description of leukemia provoked by 9:10 dimetil-1:10 benzatratsen (Nemenova and Khokhlova).

Thus, together with diffuse hyperplasis and myelosis of marrow, there is noticed an infection of the greatest part of the liver. In the latter, the growth of leukemic cells proceeds mainly in the glisson capsule and to a lesser degree along the course of the capillaries.

In the lymphatic knots, notwithstanding the intensity of the leukemic process, myelosis usually has a focal character with a preservation of the structure of the lymphoid tissue. In the spleen on the bottom of the diffuse myelosis we can also distinguish the diminishment of the follicles of the normal structure.

It also seems to be a characteristic of experimental leukemia that in the marrow as well as in the inner organs, there always are present together with unripe myeloid cells, in larger or smaller quantity, ripe elements--segmented leukocytes. And finally in a significant number of mice, leukemic changes in organs were not expressed microscopically.

The sometimes noticable significant enlargement of the liver and spleen is dependent, as was shown by the gystological examination mainly on amiloidosis. In some of these mice leukemia was absent altogether. According to the preponderance of these or otner cells in the marrow and in the internal organs, it was possible to separate the hemocytoblasts, myeloblast, or myelocytal type of leukemia, and the last was met the most frequently.

Leukemic changes in the organs usually matched the leukemic picture of blood and only in single cases was there an aleukemic variation.

Regardless of the fact that benzol extracts were introduced from organs of patients who died from various forms of leukemia-chronic myelosis, lymphadenosis, acute leukemia and also myelosis--the mice developed myeloid leukemia. It should be noted that in one case, when extract from organs of a patient who died of myelosis was introduced, there was observed in the mouse changes of the type of diffuse myelosis.

In our investigation we did not see any difference between the changes developed through the effects of extracts from different organs. The highest percentage of infection with leukemia (5.3 per cent) with intensive extramedullar blood-formation and positive results in transfers was obtained through the introduction of benzol extract from the liver and spleen of patients who died of chronic myelosis. After introduction of extracts from organs of patients who died of chronic lymphadenosis, in the original mice there was observed the appearance of tumors only, and myelosis developed only in the subsequent transfers.

It is interesting to remark that after the introduction of extracts from organs of patients who died of acute leukemia, in the midst of blood-forming cells both in the original mice and in the transfer, there always predominated most unripe elements--myeloblasts and hemocytoblasts.

In some of the mice, leukemia accompanied the development of tumors which arose usually in the place of introduction of the preparation, but sometimes at a distance from it. In the original mice, the tumor had the appearance of spindle sarcoma, which in the subsequent transfers assumed the structure of polymorpho-cellular sarcoma (ill. 4). In a small number of mice there appeared so-called distant

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[redacted] the appearance of [redacted] of the dividing wall, sarcoma of the [redacted] and adenoma of the lung, coming from the [redacted] wall.

Sometimes there was a hyperplasis of lymphoid tissue in the remainder of the thyroid gland, but without symptoms of a malignant growth. In single cases the tissue of the thyroid gland also was subject to myeloid metaplasis. In some mice in the place of introduction of the preparation there developed sarcoma without the symptoms of leukemia.

The changes which developed after the introduction of benzol extracts can be divided into three main groups. Characteristic of the first group is the presence of leukemia. In the second group, leukemia accompanied the appearance of swelling, usually in the place of introducing the extract and sometimes at a distance from it. In the third group belong the cases which were observed the most frequently, in which there was a development of a tumor, but leukemia was absent. In a part of the mice studied, there were no changes.

In the analysis of our material, it was important to work out clear morphological criteria for the diagnostic of true experimental leukemia in order to distinguish it from so-called leukimoid ractions. In the latter there are observed changes chiefly in the peripheral blood (leukocytes, the appearance of young forms), at a time when in the internal organs there is a reaction only on the part of the reticulo-endotelial system in the form of a swelling of reticular cells, and in the marrow some hyperplasis of the blood-forming tissue, on the whole without the predominance of cells of white blood; properly leukemic changes are absent.

Diagnosis of true experimental leukemia we made only in those cases where there was a diffuse infection of organs of the blood-creating system with a growth of myeloid tissue and leukemic infiltration in the inner necro-creating organs. Together with the changes described, there usually was observed also a leukemic pattern of blood.

True experimental leukemia in mice seems to be a fatal disease, at the time when leukemoid changes originating in peripheral blood are returned.

Beside morphological proof for the confirmation of true experimental leukemia, as is known, positive results of transfers are necessary. But only through pathologoanatomical examination is it possible to determine exactly the diagnosis of experimental leukemia and to distinguish it from leukoid conditions. Thus when benzol

[redacted]

[redacted] emia, and [redacted] other malignant neoplasm, [redacted] the genetic connection of the processes.

The obtaining of experimental leukemia through the introduction of extracts not only from organs of patients who died of chronic leukemia, but also from organs of patients who died of acute leukemia, indicates the common nature of these diseases. The possibility of inducing experimental leukemia both through the aid of cancerogenous material, as well as through the method of introducing benzol extracts from organs of patients who died of leukemia, allows us to consider it a distinctive tumorous process.

## Conclusions

1. Through the introduction of benzol extracts from organs of patients who died of leukemia, into mice, 4.9 per cent of cases there results the growth of myeloid tissue in the organs of the blood-forming system and outside of it; that is, there were observed pathologoanatomical changes characteristic of leukemia. In the majority of experiments these changes were accompanied by a firm leukemic picture of the blood and gave positive results in transfers, which seems to be a confirmation of the presence of true leukemia.

2. Both with the original mice and with the transfers there was obtained only a myeloid leukemia--hemocytoblastic, myeloblastic, and myelocytic types being observed. The last was met the most frequently.

3. Through the introduction of benzol extracts from organs of patients who died of chronic leukemia, mice usually developed a myelocytaric type of leukemia; in the introduction of extracts from organs of patients who died of acute leukemia, hemocytoblasts were always found.

4. The fact of contraction of experimental leukemia through the introduction of extracts not only from the organs of the patients who died of chronic leukemia, but also from the organs of patients who died of acute leukemia, indicates the identical nature of these processes.

5. In some of the mice there was observed the development of malignant tumor--usually in the place of introducing the preparation, sometimes also at a distance from it.

The received data showed that the introduction of one and the same benzol extract can bring about leukemia, leukemia accompanied by tumor, and, finally, only tumor.

[redacted]

6. Leukemia contracted through the introduction of benzol extracts of organs of patients who died of leukemia, and through the introduction of chemically pure cancerogenous materials, is identical in all morphological structure.

7. The possibility of bringing about of tumors, together with leukemia, through the introduction of both chemically pure cancerogenous materials and of benzol extracts from the organs of patients who died of leukemia, points to the pathologenetic closeness of these diseases.

## Literature

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## Microphotographs to the article of M P Khokhlova

1. Liver. Leukemia. Intensive intracapillary bloodformation.

2. Spleen. Myelosis of the pulp.

3. Marrow of the hip. Myelosis with predominance of myelocytes and the presence of segmented leukocytes.

4. Polymorphocellular sarcoma.

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